VERSION WITH MARKINGS TO SHOW CHANGES MADE IN THE SPECIFICATION

Paragraph beginning at line 10 of page 2 has been amended as follows:

Streptococcus mutans type c and type d are separately grown in BHI or TTY culture medium for 2-3 days and centrifuged to collect the bacteria. The bacteria are washed 4-6 times with 0.05-0.2 M of phosphate buffered saline, pH 6-7, heated at 50-60°C for 25-35 minutes. To prepare antigens, mix streptococcus mutans type c and type d by ratio 1:1-2:1, add Freund' s adjuvant equal to total volume of both bacteria, and then treat with high speed [homogenizer] homogenize machine.

Paragraph beginning at line 17 of page 2 has been amended as follows:

The hens are immunized by three hypodermic or wing vein injections, 1.0ml ($1x10^9$/ml) of streptococcus mutans each time, at 2weeks intervals. Yolks are taken out by sieve, stirred even, and diluted by adding 4-6 fold of distilled water. Adjust pH to 4.5-6.5, stand at 3-5°C for 20-30 hours, and centrifuge at high speed for 20-30 minutes. The supernatant is ultrafiltrated, followed by [0.22μ] 0.22μm membrane filtration to eliminate bacteria and lyophilization. This is crude IgY extract against dental caries.

Paragraph beginning at line 1 of page 3 has been amended as follows:

Obtained eluates are applied on Sephadex G200 column, and eluted with phosphate buffer containing 0.05-0.2M of NaCl by gradient elution followed by pouring protein peaks, estimating antibody activity with "ELISA", eliminating bacteria by [0.22μ] 0.22μm membrane filtration, and lyophilizing. This is purified IgY against dental caries bacteria.

Paragraph beginning at line 19 of page 5 has been amended as follows:

Three milliliter (10 mg/ml) of crude IgY extract are applied on "DEAE–Sephadex A50" column (2.5x35cm), eluted with pH 7.0, 0.01M of phosphate buffer containing 0.07M of NaC1, 20ml/h. 5.0ml each fraction. The protein peaks are poured. Antibody activity are estimated with "ELISA". Active eluates are poured. Adjusted to 20mg protein/ml. Then, 1.5ml of it is applied on "Sephadex G200" column (2.0x65cm) and eluted with pH 7.0, 0.01M of phosphate buffer containing 0.1M of NaCl, 8.0ml/h. 5.0ml each fraction. The protein peaks are poured and estimated for antibody activity

with "ELISA". Active eluates are poured, bacteria-eliminated with [0.22μ] 0.22μm membrane filtration, and then lyophilized. This is purified IgY against dental caries bacteria.

Paragraph beginning at line 1 of page 6 has been amended as follows:

Sample 2 Streptococcus mutans type c and type d are separately cultivated in TTY medium at 37°C for 48 hours, collected by centrifugation at 4000 rpm for 10 minutes, washed with pH 6.5, 0.15M of phosphate buffered saline 5 times, and heated at 65°C for 25 minutes. Then, make type c and type d suspensions, $2x10^9$/ml each. Mix [equal] 2:1 volumes of type c and type d suspensions to get mixture ($2x10^9$/ml) of them. Add Freund's adjuvant equal to the volume of the mixture. Treat it with high speed [homogenier] homogenize machine to get streptococcus mutans antigens.

Paragraph beginning at line 14 of page 6 has been amended as follows:

To get purified IgY against dental caries bacteria, apply 4.0ml (10 mg/ml) of crude IgY on "DEAE-Sephadex A50" column (2.5x35cm), elute with pH 7.0, 0.01M of phosphate buffer containing 0.06M of NaCl, 20ml/h, 5.0ml each fraction, pour each peask, estimate antibody activity with "ELISA". Keep the active eluates, eliminate bacteria by [0.22μ] 0.22μm membrane filtration and lyophilize.

Paragraph beginning at line 17 of page 7 has been amended as follows:

Add IgY of the present invention and potassium sorbate, which final concentrations are 0.1% and 0.015% respectively, into pasteurized fresh milk, homogenize with sterile [homogenized] homogenizer. Pour into sterile sucking bottles and store at 4°C.

REMARKS-General

1. Upon review of the original specification and in light of the observation of the Examiner noted in the above Office Action, the applicant has amended the specification which is deemed to more clearly and distinctly describe the subject matter of the instant invention, and which provides full antecedent basis to the newly drafted claims. No new matter has been included in the amended specification.

2. The newly drafted independent claim 28, 32 and 34 incorporates limitations previously brought forth in the disclosure. No new matter has been included. All new claims 28 to 38 are submitted to be of sufficient clarity and detail to enable a person of average skill in the art to make and use the instant invention, so as to be pursuant to 35 USC 112.

Response to Rejection of Claims 13 to 27 under 35USC112

3. The applicant submits that the newly drafted claims 28 to 38 particularly point out and distinctly claim the subject matter of the instant invention, as pursuant to 35USC112.

Response to Rejection of Claims 13 to 27 under 35USC103

4. The Examiner rejected claims 13-20 and 26 over US Pat No. 5,367,054(Lee et al) or Akita et al each in view of US Pat No. 4,324,782 or Hatta et al or Harlow et al or Hamada et al or Grassman et al and US Pat No. 4,400,376.

5. The cited art, US Pat No. 5,367,054(Lee et al), suggests a LARGE-SCALE PURIFICATION OF EGG IMMUNOGLOBULIN that emphasizes on purification of IgY with a more complicated preparation procedure. It contains several times of ion exchange and process of precipitation or gel filtration and de-salting. Obviously, the cited art only mentioned the PURIFICATION OF IgY, but the instant invention mentioned the PREPARATION OF IgY AGAINST DENTAL CARIES BACTERIA, not only the purification but also preparing streptococcus mutants antigens and immunizing hens. And in the purification of IgY, the cited art substantially differs to the instant invention in many aspects as follows:

(a) Purification of IgY in the present invention only contains applying DEAE-Sephadex A50 and Sephadex G200 once, both of the two said materials are inert substances and recyclable, no materials used in the instant invention would cause chemical pollution and the cost of preparation is low.

(b) In the extracting of crude IgY, the cited art add 1% caprylic acid in the diluted yolk when homogenize the yolk, thus after the purification of IgY, the leavings can't be reused, and will bring circumstance pollution.

6. The cited art, Akita el al, suggests Isolation and Purification IgY from Egg Yolk, it contained steps of further purified by salt precipitation, alcohol precipitation, ultrafiltration (UF), gel filtration and anion exchange chromatography. The difference between the cited art and the instant invention is the reagent used in the purification of IgY, the instant invention use DEAE-Saphadex A50 and Sephadex G200. We found the best concentrations of NaCl in phosphate buffer eluants for DEAE-Sephadex A50 column and Sephadex G200 column are 0.07M and 0.1M. The IgY of present invention has reached PAGE purity with 180,000D of molecular weight by SDS-PAGE, and its activity is good.

7. US Pat No.4,324,782 suggests extracting the Streptococcus mutants antibody from milk and cultivating the Streptococcus mutants, the technique of the cultivation is a matured technique, and it isn't the innovative points of the instant invention.

8. The cited art, Hatta et al, on the other hand, teaches a method preparation against dental carries using Streptococcus mutants serotype c, but Hatta et al didn't mention serotype d and their best ratio. Using mention serotype c and d and finding their best ratio are innovative points of the claimed invention. On the other hand, we must point out it again that the content of Hatta et al research is unrelated to its coversheet that it doesn't refer to the real content of Hatta's research, the Examiner should not quote the fault content and compare with the instant invention.

9. The cited art, Hamada et al, merely suggests how to isolate Streptococccus mutants from Japanese children and implant them in Rats' teeth to improve that the Streptococccus mutants are leading factors of the dental caries. Furthermore, Hamada et al. mentions serotypes c, d, e and f induced dental caries in SD rat. The instant invention composes the steps of cultivating streptococcus mutant antigens, immunizing

hens and purifying IgY from Egg yolk. It is obviously different from the instant invention in view of purpose, principle and content, so there is no comparability between the cited art and the instant invention.

10. The cited art, Gassmann et al, mentions efficient production of chicken egg yolk antibodies against a conserved mammalian protein. The content of the cited art does not belong to the same technical field of the instant invention, so there is no comparability between them.

11. The cited art, Harlow et al, teaches question of Freund's adjuvant. It is a well-known method to add the Freund's adjuvant in to prepare antigen, and it isn't the innovative points of the instant invention.

12. US Pat No. 4,400,376 suggests in purification of immunological preparations using Sephadex G-200 and obtained ovalbumin/antiB2M complex (molecular weight 100,000) and unreacted ovalbumin plus NEM Fab/MBS (molecular weight 50,000). The present invention also uses Sephadex G200, but the production of the two inventions is different, so there is no comparability between the two articles.

13. The Examiner rejected claims 21-25 and 27 over US Pat No. 5,367,054 (Lee et al) or Akita et al each in view of US Pat No. 4,324,782 or Hatta et al or Harlow et al or Hamada et al or Grassman et al and US Pat No. 4,400,376, and further in view of US Pat. No.4,136,094.

14. US Pat. No. 4,136,094 teaches sterilizing immunoglobulin by filtration through a 0.22μm membrane for intravenous injection and the advantages of filtration through a 0.22μm membrane.

15. In the biological field, the 0.22μm membrane is a common technology, and there is no innovation. We think that the intention of the author is to point out the advantages of the technology not the advantages of the membrane. The purpose of the membrane is to sterilize.

16. The applicant respectfully submits that the invention must be considered as a whole and there must be something in the reference that suggests the combination or the modification. See Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick,

221 U.S.P.Q. 481,488 (Fed. Cir. 1984) ("The claimed invention must be considered as a whole, and the question is whether there is something in the prior art as a whole to suggest the desirability, and thus the obviousness, of making the combination"), In re Gondon, 221 U.S.P.Q. 1125, 1127 (Fed. Cir. 1984), ("The mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification.") In re Laskowski, 10 U.S.P.Q.2d 1397, 1398 (Fed.Cir. 1989), ("Although the Commissioner suggests that [the structure in the primary prior art reference] could readily be modified to form the [claimed] structure, the mere fact that the prior art could be modified would not have made the modification obvious unless the prior art suggested the desirability of the modification.")

17. In the present case, there is no such suggestion. In any case, even combining all of the said cited articles would not provide the invention as claimed – a clear indicia of nonobviousness. Ex parte Schwartz, slip op.p.5 (BPA&1 Appeal No. 92-2629 October 28,1992), ("Even if we were to agree with the examiner that it would have been obvious to combine the reference teachings in the manner proposed, the resulting package still would not comprise zipper closure material that terminates short of the end of the one edge of the product containing area, as now claimed."). That is, modifying each of the said cited articles as proposed by the Examiner, would not provide the following features:

(a) **A long period of validity:** In the claimed invention, after first immunize hens 20th days, eggs can keep active antibody (immunoglobulin of yolk) for approximately 13 months. In current documents, this duration of existing techniques is only half a year. So, the period of validity increases two times than the current techniques.

(b) **High titer of antibody:** If existing preparative techniques are used to prepare antibody, the highest titer is only 1:320 at present. In the instant invention, the titer can be 1:512.And the ELISA estimating antibody activity and be 204800. More antibodies can be produced in the instant invention in the same circumstance.

(c) **Good effect on restraining activity of streptococcus mutants:** Research has showed that forty-eight hours after different concentrations of IgY are put into culture medium of streptococcus mutants, activity of streptococcus mutants is

restrained, and the pHs rises to different extents. This result proves that every pH is above 6.0 (Experiment proves dental caries occur only at the critical value pH 5.0~5.5 or even below). And animal experiment also further suggested that IgY can effectively protect dental caries.

(d) **No chemical pollution and comprehensively utility of remains:** There is no chemical pollution in the reparation process. After extracting, the eggshell can be made into Calcium powder, egg white can be made into peptone, the remainder of yolk can be made into food and remained yolk can be utilized as lecithin, vitalize oil and other food. There leaves on cast off, therefore there is no environmental pollution.

(e) **Low preparation cost:** In both processes of extracting crude IgY and purifying extracted IgY, the techniques are very simple and DEAE-Sephadex A50 and Sephadex G200 can be utilized repeatedly. Therefore, the whole preparation cost is relatively lower than current techniques.

(f) **Implementation probability:** The success ration of implementing the instant invention will reach 100% if the implementation strictly follows the operation of the instant invention.

(g) **High purity**: Follow the steps of the instant invention, especially use the DEAE-Sephadex A50 and Sephadex G200 can make the IgY of the present invention reached PAGE purity with 180,000D of molecular weight by SDS-PAGE. The best concentrations of NaCl in phosphate buffer eluates for DEAE-Sephadex A50 column and Sephadex G200 column are 0.07M and 0.1M respectively. In other words, we obtain above better purity because of selecting proper concentration as described above.

(h) **Dental caries-preventing combinations**: The IgY of the instant invention can makeup many different kinds of IgY productions, such as IgY buccal liquid, IgY chewing gum, IgY toothpaste, IgY tooth-protecting paste, IgY nutrient milk, IgY nutrient milk powder or IgY nutrient bean milk et al.

The Cited but Non-Applied References

18. The cited but not relied upon references have been studied and are greatly appreciated, but are deemed to be less relevant than the relied upon references.

19. In view of the above, it is submitted that the claims are in condition for allowance. Reconsideration and withdrawal of the objection and rejection are requested. Allowance of claims 28 to 38 at an early date is solicited.

Respectfully submitted,

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